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(54) Title: CALCITRIOL DERIVATIVES AND THEIR USES			
(57) Abstract A method of treating metabolic bone diseases such as osteoporosis, as well as other disease states treatable with vitamin D compounds, comprises administering an effective amount of one or more modified vitamin D compounds. The modified compound contains a typical vitamin D nucleus but with in vivo hydrolyzable groups at one or more of the 1, 3 and 25 carbon positions. The time of conversion of the modified compound to its active form, such as calcitriol, can be regulated to thus provide controlled release of the compound in vivo over time, by changing or modifying the hydrolyzable groups.			

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CALCITRIOL DERIVATIVES AND THEIR USES**Background of the Invention**

This invention relates to biologically active vitamin D compounds, and more particularly to vitamin D compounds with hydrolyzable groups at one or more of the 1, 3 and 25 carbon positions, such as esters of $1\alpha,25$ -dihydroxyvitamin D_3 or esters of $1,25$ -dihydroxyvitamin D_3 analogs, and their use to regulate over time the function of $1,25(OH)_2D_3$ (or of $1,25(OH)_2D_3$ analogs) during the treatment of a variety of diseases such as osteoporosis, renal osteodystrophy, hypoparathyroidism or proliferative skin disorders.

The 1α -hydroxylated metabolites of vitamin D -- most importantly $1\alpha,25$ -dihydroxyvitamin D_3 and $1\alpha,25$ -dihydroxyvitamin D_2 -- are known as highly potent regulators of calcium homeostasis in animals and humans. With the discovery of $1\alpha,25$ -dihydroxyvitamin D_3 as the active form of the vitamin has come an intense investigation of analogs of this hormonal form of vitamin D with the intent of finding analogs that have selective biological activity. As a consequence, many structural analogs of these metabolites, such as compounds with different side chain structures, different hydroxylation patterns, or different stereochemistry, have been prepared and tested. Important examples of such analogs are 1α -hydroxyvitamin D_3 , 1α -hydroxyvitamin D_2 , various side chain fluorinated derivatives of $1\alpha,25$ -dihydroxyvitamin D_3 , 19-nor-vitamin D compounds, and side chain homologated analogs. Several of these known compounds exhibit highly potent activity in vivo or in vitro, and some of these have been found to exhibit an interesting separation of activities in cell differentiation and calcium regulation. This difference in activity provides these compounds

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with advantageous therapeutic activity profiles and thus some of these compounds are in use, or have been proposed for use, in the treatment of variety of diseases such as renal osteodystrophy, vitamin D-resistant rickets, osteoporosis, psoriasis, and certain malignancies.

Various forms of osteoporosis are known, e.g., postmenopausal, senile and steroid-induced osteoporosis, one of the characteristics of which is the loss of bone mass. Females at the time of menopause suffer a marked loss of bone mass giving rise ultimately to osteopenia, which in turn gives rise to spontaneous crush fractures of the vertebrae and fractures of the long bones. This disease is generally known as postmenopausal osteoporosis and presents a major medical problem, both in the United States and most other countries where the life-span of females reaches ages of at least 60 and 70 years. Generally, the disease which is often accompanied by bone pain and decreased physical activity, is diagnosed by one or two vertebral crush fractures with evidence of diminished bone mass. It is known that this disease is accompanied by diminished ability to absorb calcium, decreased levels of sex hormones, especially estrogen and androgen, and a negative calcium balance.

Similar symptoms of bone loss characterize senile osteoporosis and steroid-induced osteoporosis, the latter being a recognized result of long term glucocorticoid (cortico-steroid) therapy for certain disease states.

Methods of treating osteoporosis have varied considerably but to date no totally satisfactory treatment is yet known. A conventional treatment is to administer a calcium supplement to the patient. However, calcium supplementation by itself has not been successful in preventing or curing the disease. Another

conventional treatment is the injection of sex hormones, especially estrogen, which has been reported to be effective in preventing the rapid loss of bone mass experienced in postmenopausal women. This technique, however, has been complicated by the fear of its possible carcinogenicity. Other treatments for which variable results have been reported, have included a combination of vitamin D in large doses, calcium and fluoride. The primary problem with this approach is that fluoride induces structurally unsound bone, called woven bone, and in addition, produces a number of side effects such as increased incidence of fractures and gastrointestinal reaction to the large amounts of fluoride administered. Another suggested method is to block bone resorption by injecting calcitonin or providing phosphonates.

U.S. Patent No. 4,255,596 suggests the use of various metabolites of vitamin D₃ for increasing calcium absorption and retention within the body of mammals displaying evidence of or having a physiological tendency toward loss of bone mass. The metabolites specifically named in that patent, i.e., 1 α -hydroxyvitamin D₃, 1 α -hydroxyvitamin D₂, 1 α ,25-dihydroxyvitamin D₃ (calcitriol), 1 α ,25-dihydroxyvitamin D₂ and 1,24,25-trihydroxyvitamin D₃, although capable of the activity described and claimed in that patent, can, however, also cause hypercalcemia, especially if used with the conventional calcium supplement. Calcitriol treatment has also been found to be effective in reducing bone loss in women with postmenopausal osteoporosis by increasing intestinal calcium absorption and reducing bone resorption. Aloria et al, "Calcitriol In The Treatment Of Postmenopausal Osteoporosis", Amer. Jour. of Med., Vol. 84, March, 1988, pp. 401-408. Again, however,

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Aloria et al discouraged the use of oral dosages of calcitriol due to the risk of hypercalcemia. Therefore, use of calcitriol to treat osteoporosis has not been widely accepted.

5 Another important consideration is that in vivo calcitriol is produced slowly and continuously by the kidney and thus is available throughout the day and night. When given by mouth or by injection, large amounts are available to the tissues initially but little is left after 2-4 hours due to metabolism and excretions. A process whereby calcitriol can be made available in vivo more slowly and more continuously would avoid peaks and valleys in the availability of calcitriol thereby providing an effective in vivo level of the compound over a more prolonged period of time and also avoiding or substantially reducing episodes of hypercalcemia that often result from the sudden availability of excessive amounts of the substance.

Summary of the Invention

The present invention provides a method for modulating and regulating the in vivo activity of biologically active vitamin D compounds, such as calcitriol or analogs of calcitriol. More specifically, this invention provides modified vitamin D compounds that exhibit a desirable and highly advantageous pattern of biological activity in vivo, namely, the more gradual onset and more prolonged duration of activity. As a consequence of such advantageous properties, these compounds represent novel therapeutic agents for the treatment of all diseases where vitamin D compounds have been shown effective, such as metabolic bone diseases or proliferative skin disorders (e.g. psoriasis) and they should prove especially useful for the treatment of diseases where bone formation is desired, such as osteoporosis (postmenopausal, senile, or steroid-induced), osteomalacia or renal osteodystrophy.

Structurally, the key feature of the modified vitamin D compounds having these desirable biological attributes is that they are derivatives of $1\alpha,25$ -dihydroxyvitamin D_3 , or derivatives of $1\alpha,25$ -dihydroxyvitamin D_3 analogs, in which a hydrolyzable group is attached to the hydroxy group at carbon 25 and, optionally, to any other of the hydroxy groups present in the molecule. Depending on various structural factors -- e.g. the type, size, structural complexity -- of the attached group, these derivatives are thought to hydrolyze to $1\alpha,25$ -dihydroxyvitamin D_3 , or to a $1\alpha,25$ -dihydroxyvitamin D_3 analog, at different rates in vivo, thus providing for the "slow release" of the biologically active vitamin D compound (i.e. $1,25$ -dihydroxyvitamin D_3 , or an analog thereof) in the body.

The "slow release" in vivo activity profiles of such compounds can, of course, be further modulated by the use of mixtures of derivatives (e.g. mixtures of different derivatives of $1,25$ -dihydroxyvitamin D_3 , or different derivatives of $1,25$ -dihydroxyvitamin D_3 analogs) or the use of mixtures consisting of one or more vitamin D derivative together with underivatized vitamin D compounds.

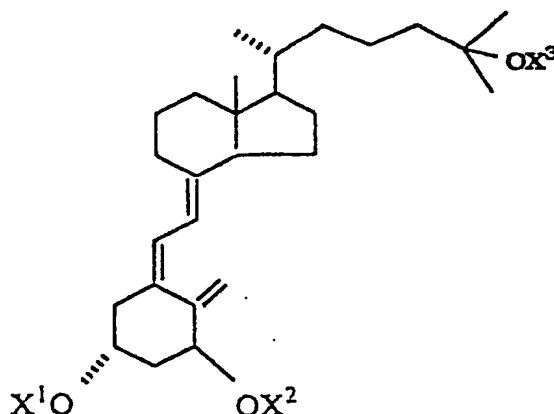
It is important to stress that the critical structural feature of the vitamin derivatives identified above is the presence of a hydrolyzable group attached to the hydroxy group at carbon 25 of the molecule. The presence of a hydrolyzable group at that position imparts on the resulting derivatives the desirable "slow-release" biological activity profile mentioned above. Other hydroxy functions occurring in the molecule (e.g. hydroxy functions at carbons 1 or 3) may be present as free hydroxy groups, or one or more of them may also be derivatized with a hydrolyzable group. The fact that the introduction of a

hydrolyzable group at carbon 25 of the vitamin D molecule markedly modulates the in vivo biological activity pattern of the resulting derivative was not appreciated previously. The realization of the importance of this specific modification, and the demonstration of its marked and highly beneficial biological effects form the basis of this invention.

The "hydrolyzable group" present in the above-mentioned derivatives is preferably an acyl group, i.e. a group of the type Q^1CO- , where Q^1 represents hydrogen or a hydrocarbon radical of from 1 to 18 carbons that may be straight chain, cyclic, branched, saturated or unsaturated. Thus, for example, the hydrocarbon radical may be a straight chain or branched alkyl group, or a straight chain or branched alkenyl group with one or more double bonds, or it may be an optionally substituted cycloalkyl or cycloalkenyl group, or an aromatic group, such as substituted or unsubstituted phenyl, benzyl or naphthyl. Especially preferred acyl groups are alkanoyl or alkenoyl groups, of which some typical examples are formyl, acetyl, propanoyl, hexanoyl, isobutyryl, 2-butenoyl, palmitoyl or oleoyl. Another suitable type of hydrolyzable group is the hydrocarbyloxycarbonyl group, i.e. a group of the type $Q^2-O-CO-$, where Q^2 is a C_1 to C_{18} hydrocarbon radical as defined above. Exemplary of such hydrocarbon radicals are methyl, ethyl, propyl, and higher straight chain or branched alkyl and alkenyl radicals, as well as aromatic hydrocarbon radicals such as phenyl or benzoyl.

Among the modified vitamin D compounds having the desirable in vivo bioactivity profile indicated above, an especially important and preferred class are certain acyl ester derivatives of calcitriol, i.e. the calcitriol derivatives characterized by the following general structure:

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where X¹ and X², independently represent hydrogen or an acyl group, and where X³ represents an acyl group as previously defined. Two other very important groups of modified vitamin D compounds are the corresponding acyl esters of the calcitriol side chain homologs, and the acyl derivatives of the 19-nor-1,25-dihydroxyvitamin D analogs.

The present invention, therefore, provides a series of modified vitamin D compounds that are useful for the treatment of metabolic bone disease (such as the various forms of osteoporosis, osteomalacia, osteodystrophy, etc.) or of differentiative diseases such as psoriasis or malignancies. More specifically, a method of treating such diseases comprises the administration of an effective amount of the above-indicated acyl ester derivatives of 1 α ,25-dihydroxyvitamin D₃ or of the corresponding derivatives of 1 α ,25-dihydroxyvitamin D₃ analogs.

The above compounds may be administered alone or in combination with other pharmaceutically acceptable agents. Dosages of from not less than about 0.5 μ g per day to not more than about 10 μ g per day of the individual compound per se, or in combinations, are generally effective. This method has the distinct advantage that it will restore bone mass due to the conversion of these compounds to calcitriol which has been

proven to be effective in the treatment of osteoporosis. Further, these compounds advantageously will be less likely to cause hypercalcemia or hypocalcemia than the underivatized compounds even if the compound is administered continuously on a daily basis, as long as the appropriate compounds and dosages are used, it being understood that the compounds and the dosage levels will be adjusted dependent upon the response of the subject as monitored by methods known to those skilled in the art.

The above method, involving the administration of the indicated dosages of these compounds is effective in restoring or maintaining bone mass, and thus provides a novel method for the treatment or prevention of various forms of osteoporosis such as postmenopausal osteoporosis, senile osteoporosis and steroid-induced osteoporosis. It will be evident that the method will find ready application for the prevention or treatment of disease states other than those named, in which the loss of bone mass is an indication. Also, it will be further evident that the method will find ready application for the prevention or treatment of hypercalcemia and hypocalcemia as the rate of conversion of these analogs, i.e. the in vivo hydroxylation process, can be controlled and regulated in the manner described above.

Brief Description of the Drawings

Fig. 1 is a graph illustrating the activity of di- and tri-acetates of $1\alpha,25$ -dihydroxyvitamin D_3 on serum calcium, and particularly illustrates the milligrams percent calcium found in blood over time; and

Fig. 2 is a graph similar to Fig. 1 except illustrating the activity of a 25-mono-acetate of $1\alpha,25$ -dihydroxyvitamin D_3 on serum calcium.

Disclosure of the Invention

The present invention provides novel modified vitamin D compounds which are useful in the treatment of metabolic bone diseases, such as osteoporosis, as well as other disease states.

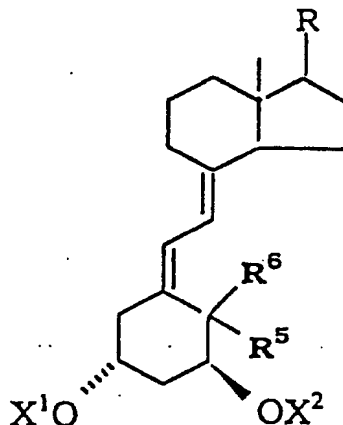
5 These modified vitamin D compounds are hydrolyzable *in vivo* to calcitriol, or analogs of calcitriol, over a period of time following administration, and as a consequence regulate the *in vivo* availability of active calcitriol, or analogs of calcitriol, thereby also modulating their activity profile *in vivo*. The term "activity
10 profile" refers to the biological response over time of vitamin D compounds such as calcitriol or analogs of calcitriol. Individual modified compounds, or mixtures of such compounds, can be administered to "fine tune" a desired time course of response.

As used herein the term "vitamin D compound"
15 encompasses compounds which have the C-ring, D-ring and 3 β -hydroxycyclohexane A-ring of vitamin D interconnected by the 5,7 diene double bond system of vitamin D together with any side chain attached to the D-ring. In other words, the vitamin D compounds encompassed herein include those having a "vitamin
20 D nucleus" comprising substituted or unsubstituted A-, C-, and D-rings interconnected by a 5, 7 diene double bond system typical of vitamin D together with a side chain attached to the D-ring. As used herein the term "modified vitamin D compound" encompasses any vitamin D compound in which one or more of
25 the hydroxy functions present in such a compound are modified by derivatization with a hydrolyzable group. A "hydrolyzable group" is a hydroxy-modifying group that can be hydrolyzed *in vivo*, so as to regenerate the free hydroxy functions.

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In the context of this disclosure, the term hydrolyzable group preferably includes acyl and hydrocarbyloxycarbonyl groups, i.e. groups of the type Q^1CO- and Q^2-O-CO , respectively, where Q^1 and Q^2 have the meaning defined earlier.

Structurally, the modified vitamin D compounds encompassed may be represented by the formula:



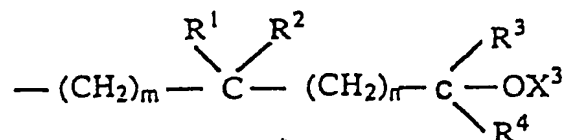
where R^5 and R^6 each represent hydrogen, or taken together R^5 and R^6 represent a methylene group.

The side chain group R in the above-shown structure represents a steroid side chain of the structure below:



where the stereochemical center (corresponding to C-20 in steroid numbering) may have the R or S configuration, (i.e. either the natural configuration about carbon 20 or the opposite unnatural configuration), and where Z is selected from Y, $-OY$, $-CH_2OY$, $-C \equiv CY$ and $-CH = CHY$, where the double bond may have the *cis* or *trans* geometry, and where Y is selected from a radical of the structure:

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- 5 where m and n, independently, represent the integers from 0 to 5, where R¹ is selected from hydrogen, OX⁴, fluoro, trifluoromethyl, and C₁₋₅-alkyl, which may be straight chain or branched and, optionally, bear a hydroxy substituent, and where R² is selected from hydrogen, fluoro, trifluoromethyl and C₁₋₅ alkyl, which may be straight-chain or branched, and optionally, bear a hydroxy substituent, and where R³ and R⁴, independently represent trifluoromethyl or C₁₋₅ alkyl, which may be straight chain or branched and, optionally, bear a hydroxy substituent, and where R¹ and R², taken together, represent an oxo group, or an alkylidene group, =CR²R², or =CR²R³, or the group -(CH₂)_p-, where p is an integer from 2 to 5, and where R₃ and R₄, taken together, represent the group -(CH₂)_q-, where q is an integer from 2 to 5. In the above-shown structures X¹, X² and X⁴ independently represent hydrogen, an acyl group or a hydrocarbyloxycarbonyl group, and X³ represents an acyl group or a hydrocarbyloxycarbonyl group, as previously defined herein.
- 20

Some specific examples of such modified vitamin D compounds include calcitriol derivatives such as:

- 25 1 α ,25(OH)₂-D₃-1,3,25-Triacetate where
X¹=X²=X³=CH₃CO;

1 α ,25(OH)₂-D₃-1,3,25-Trihexanoate where
X¹=X²=X³=CH₃(CH₂)₄CO;

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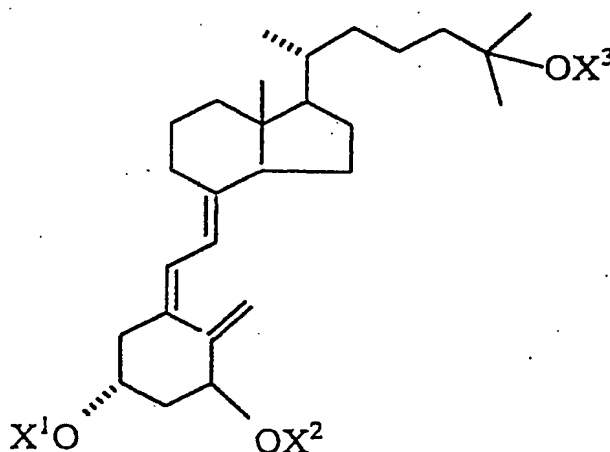
$1\alpha,25(\text{OH})_2\text{-D}_3\text{-1,3,25-Trinonanoate}$ where
 $\text{X}^1=\text{X}^2=\text{X}^3=\text{CH}_3(\text{CH}_2)_7\text{CO};$

$1\alpha,25(\text{OH})_2\text{-D}_3\text{-25-Acetate}$ where $\text{X}^1=\text{X}^2=\text{H}$ and
 $\text{X}^3=\text{CH}_3\text{CO}.$

5

The following examples, which are meant to be illustrative only, describe the process of synthesis of modified vitamin D compounds. In these examples, specific compounds identified by Arabic numerals (e.g. compounds 1, 2, 3, . . . etc.) refer to the structures so numbered below. Additionally, examples are provided which are illustrative of the distinctive biological characteristics of the new compounds, such characteristics serving as a basis for the application of these compounds in the treatment of metabolic bone disease and other vitamin D responsive diseases such as psoriasis.

20



25

- 1: $\text{X}^1 = \text{X}^2 = \text{X}^3 = \text{H}$
 2: $\text{X}^1 = \text{X}^2 = \text{X}^3 = \text{CH}_3\text{CO}$
 3: $\text{X}^1 = \text{X}^2 = \text{X}^3 = \text{CH}_3(\text{CH}_2)_4\text{CO}$
 4: $\text{X}^1 = \text{X}^2 = \text{X}^3 = \text{CH}_3(\text{CH}_2)_7\text{CO}$
 5: $\text{X}^1 = \text{X}^2 = (\text{CH}_3)_2\text{CHCO}; \text{X}^3 = \text{H}$

30

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6: $X^1 = X^2 = \text{CH}_3(\text{CH}_2)_4\text{CO}$; $X^3 = \text{H}$

7: $X^1 = X^2 = \text{C}_6\text{H}_5\text{CO}$; $X^3 = \text{H}$

8: $X^1 = X^2 = \text{H}$; $X^3 = \text{CH}_3\text{CO}$

5 EXAMPLE 1

The Preparation of Mono, Di and Tri-Esters of $1\alpha,25\text{-(OH)}_2\text{-D}_3$

Experimental

10 Spectra were obtained on the following instruments: Ultraviolet (UV) absorption spectra were taken with Perkin-Elmer Lambda 3B UV/vis spectrophotometer. Nuclear magnetic resonance (NMR) spectra were recorded at 400 or 500 MHz with a Bruker DMX spectrometer. Chemical shifts (δ) are reported downfield from internal Me_4Si (δ 0.00). Mass spectra were recorded at 70
15 eV on a Kratos MS-50 TC instrument equipped with a Kratos DS-55 data system. Preparative thin layer chromatography (p.l.c.) was carried out with Merk 1-mm F-254 silica gel plates and E. Merk 0.25 mm F-254 silica gel plates.

20 **General Procedure for Preparation of Triesters:** Preparation of $1\alpha,25\text{-(OH)}_2\text{-D}_3$ 1,3,25-Triacetate (2) $X^1 = X^2 = X^3 = \text{CH}_3\text{CO}$. A solution of glacial acetic acid (0.05mL, 0.87mmol). trifluoroacetic anhydride (0.05mL, 0.35mmol) was stirred at room temperature 3.5 h. Then $1\alpha,25\text{-(OH)}_2\text{-D}_3$ (1) (0.3 mg, 0.00072mmol) in 0.4
25 mL dry THF was added to above solution at 0°C . The reaction mixture was stirred at 0°C for 0.5h and then at room temperature for 2h. The solvent was removed under reduced pressure to afford a crude product that was purified twice by preparative thin layer chromatography (p.l.c., 15%
30 EtOAc/hexane) to give a pure compound (2) (0.122mg, 31.3% yield). UV, λ max 265 nm (Et OH). ^1H NMR (CDCl_3): δ , 0.51 (s,

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3H, C₁₈-CH₃), 0.92(d, J=5.0 Hz, 3H, C₂₁-CH₃), 1.42 (s, 6H, C_{26,27}-CH₃), 1.98, 2.04, 2.06 (s, 9H, CH₃CO-), 5.04 (s, 1H, C₁₉ E-H), 5.18 (m, 1H, C₃-H), 5.31 (s, 1H, C₁₉ Z-H), 5.48 (t, J=6 Hz, C₁-H), 5.91 (6.35 (dd, J=10 Hz, C_{6,7}-H), MS: m/z 542 (M⁺) 482 (M⁺ -60), 422 (M⁺ -2 x 60), 362 (M⁺ 3 x 60).

10 1 α ,25 (OH)₂-D₃ 1,3,25-Trihexanoate (3) X¹=X²=X³=CH₃(CH₂)₄ CO. To hexanoic acid (0.15mL, 1.20mmol) was added trifluoroacetic anhydride (0.1mL 0.71mmol). The mixture was stirred at room temperature for 4h (the solution became pale brown). After 1 α ,25(OH)₂-D₃ (1) (0.4 mg, 0.00096mmol) in 0.2mL dry THF was added, the reaction mixture was stirred at room temperature for 5h, or until complete (as monitored by thin layer chromatography). The product (3) (0.217mg, 31.72% yield) was worked-up as in the previous example and purified by p.l.c. (3 times; 8% EtOAc/Skellysolve B). UV, λ max 265.3nm, 245nm (shoulder) (EtOH). ¹H NMR (CDCl₃): δ , 0.51, (s, 3H, C₁₈-CH₃), 0.89 (t, J=6.3 Hz, 9H, CH₃(CH₂)₄ CO), 0.92 (d, J=6.3 Hz, 3H, C₂₁-CH₃), 1.42 (s, 6H, C_{26,27}-CH₃), 5.14 (s, 1H, C₁₉ E-H), 5.18 (m, 1H, C₃-H), 5.32 (s, 1H, C₁₉ Z-H), 5.47 (t, J=6.3 Hz, 1H, C₁-H), 5.91, 6.34 (dd, J=11.15 Hz C_{6,7}-H), MS: m/z 710 (M⁺), 594 (M⁺-116 C₅H₁₁COOH), 478 (M⁺-2 x 116), 362 (M⁺-3 x 116).

25 1 α ,25(OH)₂-D₃ 1,3,25-Trinonanoate (4) X¹=X²=X³=CH₃(CH₂)₇CO. To nonanoic acid (0.2mL, 1.145mmol) was added trifluoroacetic anhydride (0.08mL, 0.566mmol), and the mixture was stirred at room temperature for 4h. After 1 α ,25(OH)₂-D₃ (1) (0.4mg, 0.00096mmol) in dry THF (0.2mL) was added, the reaction mixture was stirred at room temperature for 4h, or until

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complete (as determined by TLC). The usual work-up gave product (4) (0.283mg, 35.2% yield). UV, λ max 265.3 nm 243.9nm (shoulder) (EtOH); ^1H NMR (CDCl_3): δ , 0.51 (s, 3H, $\text{C}_{18}\text{-CH}_3$), 0.88 (t, $J=6.2$ Hz, 9H, $\text{CH}_3(\text{CH}_2)_7\text{CO-}$), 0.92 (d, $J=6.3$ Hz, 3H, $\text{C}_{21}\text{-CH}_3$), 1.42 (s, 6H, $\text{C}_{26,27}\text{-CH}_3$), 5.04 (s, 1H, C_{19} E-H), 5.18 (m, 1H, $\text{C}_3\text{-H}$), 5.32 (s, 1H, C_{19} Z-H), 5.49 (t, $J=6.2$ Hz, 1H, $\text{C}_1\text{-H}$), 5.91, 6.34 (dd, $J=11.36$ Hz, 2H, $\text{C}_{6,7}\text{-H}$). MS: m/z 836 (M^+), 678 (M^+-158 , $\text{CH}_3(\text{CH}_2)_7\text{COOH}$), 520 ($\text{M}^+-2 \times 158$), 362 ($\text{M}^+-3 \times 158$).

General Procedure for Preparation of Diesters: Preparation of $1\alpha,25(\text{OH})_2\text{-D}_3$ 1,3-diisobutyrate (5) $\text{X}^1=\text{X}^2=(\text{CH}_3)_2\text{CHCO}$; $\text{X}^3=\text{H}$;

A solution of isobutyric acid (0.06mL, 0.647mmol), N,N-dicyclohexylcarbodiimide (35.55mg, 0.173mmol), 4-pyrrolidinopyridine (6.12mg, 0.041mmol) and $1\alpha,25(\text{OH})_2\text{-D}_3$ (1) (0.4mg, 0.00096mmol) in 0.15mL dry dichloromethane was stirred at room temperature overnight (12h). The white precipitate formed was filtered and the residue washed with dichloromethane. The combined organic solution was concentrated and the crude product was purified twice by p.l.c. (25% EtOAc/skellysove B) to give a pure product (5) (296mg, 55.33% yield). UV, λ max 266.5nm, 244nm (shoulder); ^1H NMR (CDCl_3): δ , 0.49 (s, 3H, $\text{C}_{18}\text{-CH}_3$), 0.93 (d, $J=6.25$ Hz, 3H, $\text{C}_{21}\text{-CH}_3$), 1.14 (t, $J=6.3$ Hz, 12H, $(\text{CH}_3)_2\text{CHCO-}$), 1.21 (s, 6H, $\text{C}_{26,27}\text{-CH}_3$), 5.05 (s, C_{19} E-H), 5.17 (m, 1H, $\text{C}_3\text{-H}$), 5.35 (s, 1H, C_{19} Z-H), 5.5 (t, $J=6.3$ Hz, 1H, $\text{C}_1\text{-H}$), 5.91, 6.35 (dd, $J=11.35$ Hz, 2H, $\text{C}_{6,7}\text{-H}$) MS: m/z 556 (M^+), 468 (M^+-88 , $(\text{CH}_3)_2\text{CHCOOH}$), 380 ($\text{M}^+-2 \times 88$), 362 ($\text{M}^+-2 \times 88 - \text{H}_2\text{O}$).

1 α ,25(OH)₂-D₃ 1,3-Dihexanoate (6), X¹=X²=CH₃(CH₂)₄CO, X³=H.

A solution of n-hexanoic acid (0.15mL, 1.19mmol), N,N-dicyclohexylcarbodiimide (73.66mg, 0.358mmol), 4-pyrrolidinopyridine (4.45mg, 0.03mmol), and 1 α ,25(OH)₂-D₃ (1) (0.5mg, 0.0012mmol) in 0.1mL dry dichloromethane was stirred at room temperature overnight (12h) After work-up as in the preceding example, the crude product was purified by p.l.c. (3 times, 15% EtOAc/skellysolve B) to give pure compound (6) (0.079mg, 10.74% yield). UV, λ max 263.6nm, 240nm (EtOH).

¹H NMR (CDCl₃): δ , 0.51 (s, 3H, C₁₈-CH₃), 0.89 (t, J=6.4 Hz, 6H, CH₃(CH₂)₄CO-), 0.93 (d, J=6.25 Hz, 3H, C₂₁-CH₃), 1.21 (s, 6H, C_{26,27}-CH₃), 5.04 (s, 1H, C₁₉ E-H), 5.18 (m, 1H, C₃-H), 5.30 (s, 1H, C₁₉ Z-H), 5.49 (t, J=6.2 Hz, 1H, C₁-H), 5.92, 6.34 (dd, J=11.26, 2H, C_{6,7}-H). MS: m/z 612 (M⁺), 496 (M⁺-116, CH₃(CH₂)₄COOH), 478 (M⁺-116-H₂O), 380 (M⁺-2 x 116), 362 (M⁺-2 x 116-H₂O).

1 α ,25(OH)₂-D₃ 1,3-Dibenzoate (7) X¹=X²=C₆H₅CO, X³=H. A

solution of benzoic acid (15.53mg, 0.127mmol), N,N-dicyclohexylcarbodiimide (42.64mg, 0.206mmol), 4-pyrrolidinopyridine (2.13mg, 0.0144mmol), and 1 α ,25(OH)₂-D₃ (1) (0.51mg, 0.00123mmol) in dry dichloromethane (0.4mL) was stirred at room temperature for 16h. After work-up as above, the crude product was purified by p.l.c. (2 times, 8% EtOAc/skellysolve B) to give a pure compound (7) (0.123mg, 16.4% yield). UV, λ max 265.4 nm, 230.5nm (EtOH), ¹H NMR (CDCl₃) δ , 0.28 (s, 3H, C₁₈-CH₃), 0.91 (d, J=5.0 Hz, C₂₁-CH₃), 1.21 (s, 6H, C_{26,27}-CH₃), 5.13 (s, 1H, C₁₉ E-H), 5.47 (s, 1H, C₁₉ Z-H), 5.51 (m, 1H, C₃-H), 5.82 (t, J=6.2 Hz, 1H, C₁-H), 5.93,

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6.45 (dd, $J=10$, $C_{6,7}\text{-H}$), 7.41, 7.54, 8.04 (m, 10 H, Ar-H). MS: m/z 624 (M^+), 606 ($M^+ - H_2O$), 502 ($M^+ - 122$ C_6H_5COOH), 380 ($M^+ - 2 \times 122$), 362 ($M^+ - 2 \times 122 - H_2O$).

5 $1\alpha,25(OH)_2\text{-D}_3$ 25-Acetate (8) $X^1=X^2=H$, $X^3=CH_3CO$.

$1\alpha,25(OH)_2\text{-D}_3$ 1,3,25-Triacetate (2) (0.168 mg, 0.00031mmol) was dissolved in 1mL of 0.6% methanolic solution of potassium carbonate. After the mixture was stirred at room temperature for 2h (or until complete by TLC), the solvent was removed under reduced pressure. The crude product was purified by p.l.c. (50% EtOAc/hexane) to give the pure product (8) (0.056mg, 39.4% yield). UV, λ max 265nm (EtOH), 1H NMR ($CDCl_3$); δ , 0.54 (s, 3H, $C_{18}\text{-CH}_3$), 0.92 (d, $J=5$ Hz, $C_{21}\text{-CH}_3$), 1.42 (s, 6H, $C_{26,27}\text{-CH}_3$), 1.98 (s, 3H, CH_3CO), 4.24 (m, 1H, $C_3\text{-H}$), 4.44 (t, $J=6$ Hz, $C_1\text{-H}$), 5.01 (s, 1H, $C_{19}\text{ E-H}$), 5.34 (s, 1H, $C_{19}\text{ Z-H}$), 6.02, 6.39 (dd, $J=10$ Hz, 2H, $C_{6,7}\text{-H}$). MS: m/z 458 (M^+), 440 ($M^+ - H_2O$), 422 ($M^+ - 2 \times H_2O$), 398 ($M^+ - 60, CH_3COOH$), 380 ($M^+ - 60 - H_2O$), 362 ($M^+ - 60 - 2 \times H_2O$).

20 EXAMPLE 2

This example illustrates the serum calcium response of rats over time to three compounds, namely, $1\alpha,25(OH)_2D_3$ -1,3,25-triacetate, $1\alpha,25(OH)_2D_3$ -1,3-diacetate, and $1\alpha,25(OH)_2D_3$ (unesterified).

25 In this biological test, rats were fed a calcium-containing (0.47% calcium), vitamin D-deficient diet for a period of 8 weeks to deplete them of vitamin D. They were then provided a single oral dose of 1,000 pmol or 1 nanomole of each of the compounds, and serum calcium was determined by bleeding the

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rats at the various times as shown in Table 1. Figure 1 is a graph illustrating the data of Table 1. Because calcium is present in the diet and hence their intestine, the rise in serum calcium largely represents intestinal calcium absorption. The results show clearly that the 1,3-diacetate and un-esterified calcitriol produce essentially the same time course of response, illustrating that acetylation of the C-1 and C-5 hydroxy groups does not significantly alter the biological response, presumably because the acyl groups at these positions are removed rapidly, e.g. by digestive enzymes. In marked contrast, the triacetate did not begin to show a response until 12 to 18 hours post-dose, peaking at 24 hours. Thus, the 25-acetate group probably remained intact over a more prolonged period and was then slowly hydrolyzed inside the body. Thus, the triacetate clearly delays utilization of the calcitriol indicating that the in vivo activity profile of the parent calcitriol can be changed very markedly by acylation of the C-25-hydroxy group.

TABLE 1

		(A)	(B)	(C)
<u>Time</u>	<u>Control</u>	<u>1,25(OH)₂D₃</u>	<u>Diacetate</u>	<u>Triacetate</u>
0 Hours	4.3 ± 0.07			
8 Hours		6.06 ± 0.89	5.42 ± 0.90	4.06 ± 0.38
12 Hours		7.39 ± 0.10	6.50 ± 0.13	4.70 ± 0.12
18 Hours		6.97 ± 0.51	6.24 ± 0.51	5.91 ± 0.30
24 Hours		6.45 ± 0.50	5.98 ± 0.43	6.7 ± 0.54
48 Hours	4.43 ± 0.18	5.59 ± 0.80	5.11 ± 0.48	5.10 ± 0.43

All data = Mean ± S.D.

(A) from (B), N.S. diff.

(A) from (C), at 8 hrs, 12 hrs, p<0.001

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EXAMPLE 3

This example illustrates the serum calcium response of rats over time to two compounds, namely, $1\alpha,25(\text{OH})_2\text{D}_3$ (unesterified) and $1\alpha,25(\text{OH})_2\text{D}_3$ -25-acetate, administered by three different methods, namely, orally (oral), intramuscularly (I.M.), and subcutaneously (Sub. Cu.).

In this test, 1 nanomole of each compound was given to vitamin D deficient rats fed a 0.47% calcium, 0.3% P diet in 0.1 ml propylene glycol 95%/5% ethanol. There were at least 4 rats per group. Serum calcium was determined at the various times shown in Table 2 following a single dose given by the indicated route. Figure 2 is a graph illustrating the data of Table 2. The results are in accord with those of Example 2. Regardless of route of administration $1,25(\text{OH})_2\text{D}_3$ -25-acetate shows a more gradual onset of in vivo activity and a delayed peak of activity. Thus, the 25-monoacetate clearly delays utilization of the calcitriol confirming that the presence of a C-25-O-acyl group has a pronounced effect on the activity pattern and time course of response of a biologically active vitamin D compound.

TABLE 2
Serum Calcium Response to
 $1,25(\text{OH})_2\text{D}_3$ and its 25-Acetate

Compound	Admini- stration Route	Serum Calcium (mg/100 ml)			
		Day 1	Day 3	Day 6	Day 10
None (Control)		5.9±0.34	-	5.7±0.34	5.2±0.63
$1,25(\text{OH})_2\text{D}_3$	Oral	9.33±0.41	-	8.6±0.52	7.15±0.68
$1,25(\text{OH})_2\text{D}_3$ 25-Acetate	Oral	7.70±0.11	8.87±0.6	8.54±0.15	8.83±0.71
"	I.M.	8.10±0.24	9.17±0.53	9.77±0.3	-
"	Sub. Cu.	7.78±0.28	9.00±0.28	9.84±0.39	-

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The modified vitamin D compound or combinations thereof can be readily administered as sterile parenteral solutions by injection or intravenously, or by alimentary canal in the form of oral dosages, or trans-dermally, or by suppository.

5 Doses of from about 0.5 micrograms to about 10 micrograms per day or modified vitamin D compound per se, or in combination with other modified vitamin D compounds, the proportions of each of the compounds in the combination being dependent upon the particular disease state being addressed and the degree

10 of bone mineralization and/or bone mobilization desired, are generally effective to practice the present invention. In all cases sufficient amounts of the compound should be used to restore bone mass. Amounts in excess of about 10 micrograms per day of modified vitamin D compound, or the combination of that

15 compound with other modified vitamin D compounds, are generally unnecessary to achieve the desired results, may result in hypercalcemia, and may not be an economically sound practice. In practice the higher doses are used where therapeutic treatment of a disease state is the desired end while

20 the lower doses are generally used for prophylactic purposes, it being understood that the specific dosage administered in any given case will be adjusted in accordance with the specific compounds being administered, the disease to be treated, the condition of the subject and the other relevant medical facts that

25 may modify the activity of the drug or the response of the subject, as is well known by those skilled in the art. In general, either a single daily dose or divided daily dosages may be employed, as is well known in the art.

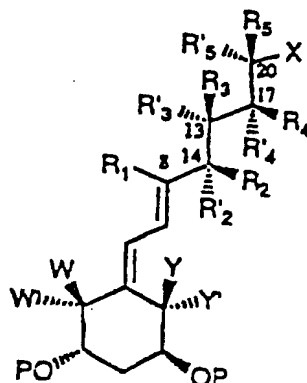
Dosage forms of the various compounds can be prepared

30 by combining them with non-toxic pharmaceutically acceptable carriers to make either immediate release or slow release formulations, as is well known in the art. Such carriers may be either solid or liquid such as, for example, corn starch, lactose,

- 21 -

- 5 sucrose, peanut oil, olive oil, sesame oil and propylene glycol. If a solid carrier is used, the dosage form of the compounds may be tablets, capsules, powders, troches or lozenges. If a liquid carrier is used, soft gelatin capsules, or syrup or liquid suspensions, emulsions or solutions may be the dosage form. The dosage form may also contain adjuvants, such as preserving, stabilizing, wetting or emulsifying agents, solution promoters, etc. They may also contain other therapeutically valuable substances.

The modified vitamin D compounds may also include any of the following compounds in which one or more hydroxy functions that may be present are modified by derivatization with a group hydrolyzable *in vivo*.



- P stands for hydrogen, alkyl or acyl;
- X represents part of the side-chain of vitamin D or of one of its established analogues;
- Y and Y', which may be the same or different, stand for hydrogen or alkyl or, when taken together, represent an alkylidene group, or form a carbocyclic ring;
- W and W', which may be the same or different, stand for hydrogen or alkyl or, when taken together, represent an alkylidene group, or form a carbocyclic ring;
- one of the carbon atoms of the central part corresponding to positions 14, 13, 17 or 20, together with the R and R' substituents connected to it, may be replaced by an oxygen (O), a sulfur (S) or a nitrogen bearing an R substituent (NR).
- R and R' (i.e., R, R₁, R₂, R'₂, R₃, R'₃, R₄, R'₄, R₅, R'₅) :
 - when located in a relative 1,3-position along the central chain, such as R₁ and R₃ or R'₃, R₂ or R'₂ and R₄ or R'₄, R₃ or R'₃ and R₅ or R'₅, taken

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together with three adjacent atoms of the central chain, which correspond to positions 8, 14, 13 or 14, 13, 17 or 13, 17, 20, respectively, can form a saturated or unsaturated carbocyclic or heterocyclic 3-, 4-, 5-, 6- or 7-membered ring

- 5 also including cases whereby geminal substituted R and R' taken together form a cyclic unsaturated bond, under the proviso that when R₁ and R'₃ form a 6-membered carbocyclic ring of the following nature (1) unsubstituted and saturated (2) monosubstituted at C-11 or (3) having a double bond between C-9 and C-11, R₂ and R₄ do not form
- 10 a five-membered carbocyclic ring when R₃ is methyl, ethyl or ethenyl
- when located in a relative 1,2-position (i.e., vicinal) along the central chain, such as R₁ and R₂ or R'₂, R₂ or R'₂ and R₃ or R'₃, R₃ or R'₃ and R₄ or R'₄, R₄ or R'₄ and R₅ or R'₅, and when not being part of a ring as described above, taken together with two adjacent atoms of the central
- 15 chain, which correspond to positions 8,14 or 14,13 or 13,17 or 17,20, respectively, can form a saturated or unsaturated carbocyclic or heterocyclic 3-, 4-, 5-, 6- or 7-membered ring, also including cases whereby geminal substituted R and R' taken together form a cyclic unsaturated bond.
- 20 • when located in a relative 1,1-position (i.e., geminal) along the central chain, such as R₂ and R'₂, or R₃ and R'₃, or R₄ and R'₄ or R₅ and R'₅, and when not being part of a ring as described above, taken together with the carbon bearing the R and R' substituents can form either a saturated or unsaturated carbocyclic or heterocyclic 3-, 4-, 5-, or
- 25 6-membered ring.
- which may be the same or different, and when they are not forming a ring or a bond as described above, stand for hydrogen or a lower alkyl group, or when taken together in the case of geminal substitution represent a lower alkylidene group.

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In the context of the invention the expression "lower alkyl group" indicates a straight or branched saturated or unsaturated carbon chain containing from 1 to 7 carbon atoms, and "lower alkylidene group" indicates a straight or branched saturated or unsaturated carbon chain containing from 1 to 7 carbon atoms, which is connected to one of the main chain atoms 14, 13, 17 and/or 20 through a double bond.

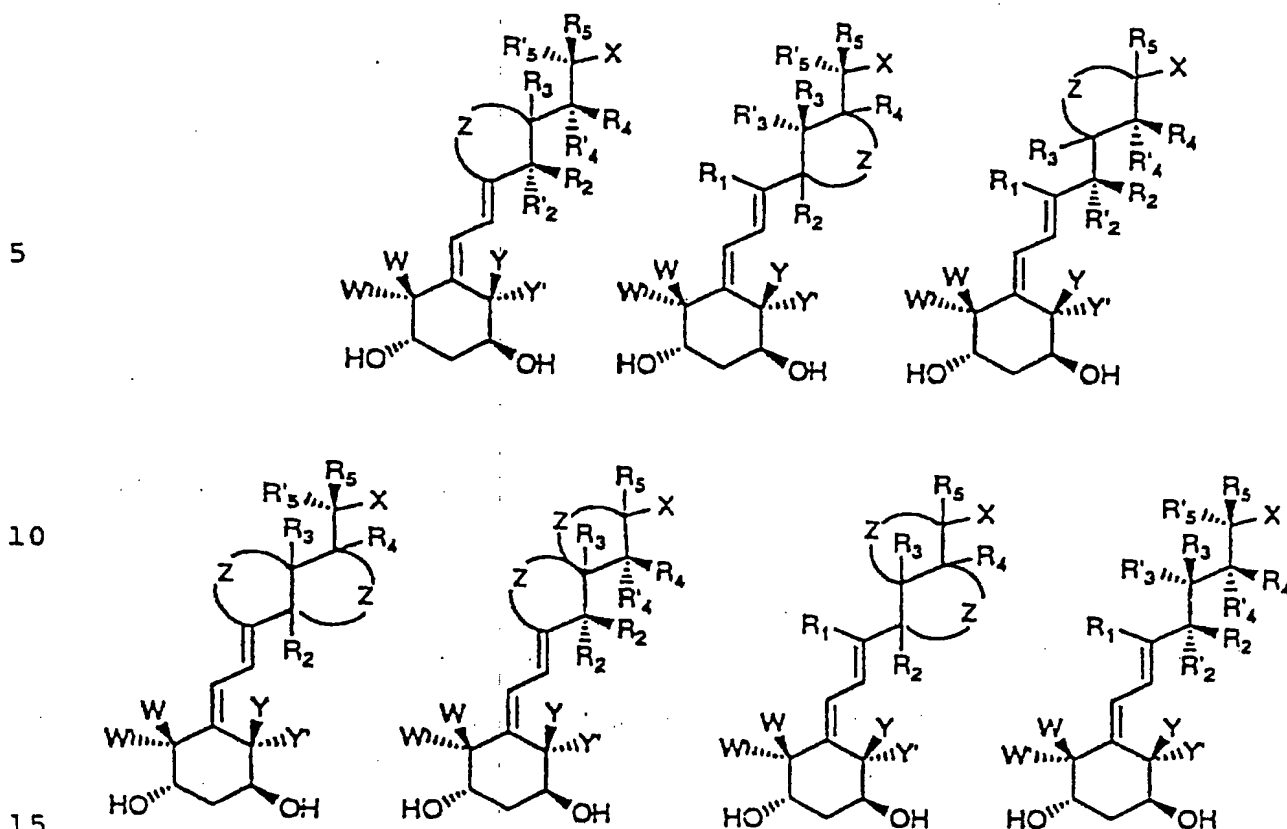
In the context of the invention part of the side-chain of vitamin D or of one of its established analogues stands for a 2 to 15 carbon atom substituted alkyl chain especially as present in vitamin D₂ (C-22 to C-28) or D₃ (C-22 to C-27) or partially modified as shown below with the vitamin D numbering, especially :

- hydroxyl substituent at one or more positions, for instance 24, 25 and/or 26 and/or
- methyl or ethyl substituent in one or more positions, for instance 24, 26 and/or 27 and/or
- halogen substituent(s) at one or more positions for instance perfluorated at positions 26 and/or 27 or difluorated at position 24 and/or
- additional carbon atom(s) especially C₂₄ between the positions 24 and 25, with the same substitution pattern mentioned above and/or
- esters derivatives of one or more hydroxyl substituents mentioned above and/or
- changing one or more carbon atoms for an oxygen, nitrogen or sulfur atom for instance at the positions 22, 23 or 24 and/or
- cyclized between the carbon atoms 26 and 27 by one bond (cyclopropane) or by the intermediacy of 1 to 4 carbon atoms, the ring can be saturated, unsaturated or aromatic and may optionally be substituted at any possible position(s) with the substituent mentioned above and/or

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- cyclized between the carbon atoms 26 and 27 by 1 to 4 atoms to form a heterocyclic ring, including aromatic, which may optionally be substituted at any possible position with the substituent mentioned above and/or
- unsaturated with one or more double or triple C-C bond(s), these
5 unsaturated chains may be substituted at any possible position by the substituents mentioned above and/or
- epoxide function can be present between carbon atoms 22,23 or 23,24 or 24,25 or 25,26; these epoxidized chains can be saturated or unsaturated and may be substituted at any possible positions with the substituents
10 mentioned above and/or
- two or more of the carbon atoms of the side chain can be linked by a single bond or by the intermediacy of a one to five carbon or oxygen, nitrogen or sulfur atoms to form a 3-7 membered saturated or unsaturated carbocyclic or heterocyclic including aromatic ring which may optimally be substituted at
15 any possible position by substituents mentioned above and/or
- substituted at one or more positions by saturated, unsaturated carbocyclic, heterocyclic or aromatic ring which can be substituted at any possible position(s) with the substituents mentioned above
- isomeric forms of the substituted chain.

20 Hence the invention relates to a series of analogues with widely varying structures. Most often the compounds of the invention are represented by one of the formulas

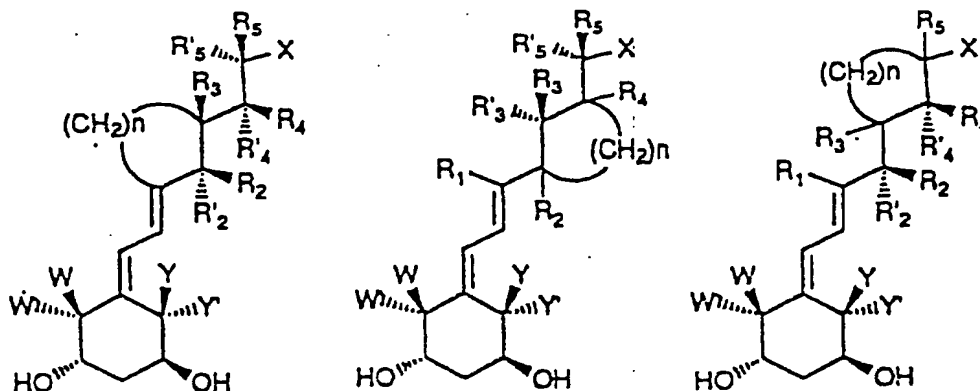


where :

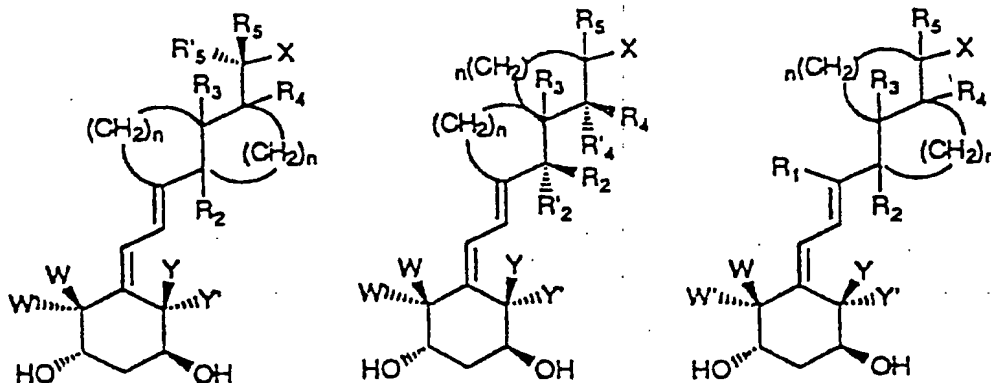
- X, Y, Y', W and W' have the same meaning as above;
- Z represents a saturated or unsaturated hydrocarbon chain consisting of zero (hence Z represents a bond between two 1,3-related carbon atoms of the central chain), one, two, three or four atoms, which may all be substituted and/or replaced by a heteroatom such as oxygen, sulfur and nitrogen.
- R₁, R₂, R'₂, R₃, R'₃, R₄, R'₄, R₅, R'₅
 - which may be the same or different, stand for hydrogen or lower alkyl, such as methyl, ethyl or n-propyl.

Among those are preferred the cyclic derivatives of type

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wherein :

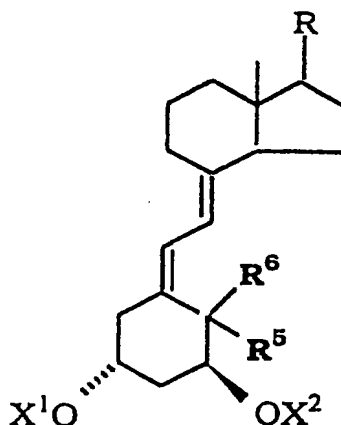
- n is an integer equal to 2 or 3;
- X represents one of the following vitamin D side-chain parts : (4-hydroxy-4-methyl)pentyl, (R)- or (S)-(3-hydroxy-4-methyl)pentyl, (3'-hydroxy-3'-methyl)butyloxy, (4-hydroxy-4-ethyl)hexyl, (4-hydroxy-4-methyl)-2-pentynyl, (4'-hydroxy-4'-ethyl)hexyloxy; 4,5-epoxy, 4-methyl-2-pentynyl; 4-hydroxy-4-ethyl-2-hexynyl; (3-methyl-2,3-epoxy)-butyloxy; (3-hydroxy-3-ethyl)-pentyloxy; (4-hydroxy-4-ethyl)-hexyloxy
- Y, Y', W and W' are the same and represent hydrogen, or taken together represent a methylene group =CH₂;
- R₁, R₂, R'₂, R₃, R'₃, R₄, R'₄, R₅ and R'₅, which may be the same or different, stand for hydrogen or methyl.

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We claim:

1. A vitamin D derivative providing for the gradual in vivo release of a biologically active vitamin D compound and thereby regulating the in vivo availability and activity profile of the biologically active vitamin D compound, said vitamin D derivative having the structure:



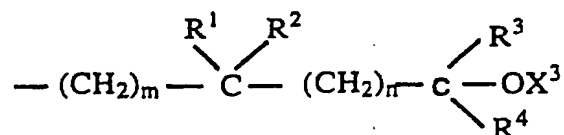
where R⁵ and R⁶ each represent hydrogen, or taken together R⁵ and R⁶ represent a methylene group, and where R is represented by the structure:



where the stereochemical center at carbon 20 in the side chain may have the R or S configuration, and where Z is selected from Y, -OY, -CH₂OY, -C ≡ CY and -CH = CHY, where the double bond may have the *cis* or *trans* stereochemical configuration, and where Y is selected from a radical of the structure:

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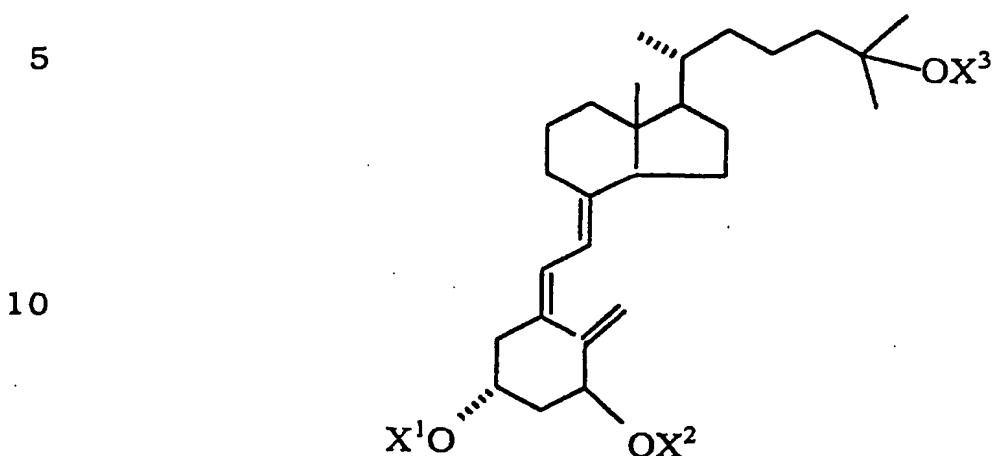
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where m and n, independently, represent the integers from 0 to 5, where R¹ is selected from the group consisting of hydrogen, OX⁴, fluoro, trifluoromethyl, and C₁₋₅- alkyl, which may be straight chain or branched and, optionally, bear a hydroxy substituent, and where R² is selected from the group consisting of hydrogen, fluoro, trifluoromethyl and C₁₋₅ alkyl, which may be straight-chain or branched, and optionally, bear a hydroxy substituent, and where each of R³ and R⁴, is selected from trifluoromethyl and C₁₋₅ alkyl, which may be straight chain or branched and optionally bear a hydroxy substituent, and where R¹ and R², taken together, represent an oxo group, or an alkylidene group, =CR²R², =CR²R³, or the group -(CH₂)_p-, where p is an integer from 2 to 5, and where R₃ and R₄, taken together, represent the group -(CH₂)_q-, where q is an integer from 2 to 5, and where X¹, X², and X⁴ are each hydrogen, an acyl group, or a hydrocarbyloxycarbonyl group, and where X³ represents an acyl group or a hydrocarbyloxycarbonyl group.

2. A vitamin D derivative according to claim 1 exhibiting a biologically active profile in vivo characterized by delayed onset or more prolonged duration of activity.

3. A calcitriol derivative providing for the gradual release of active calcitriol in vivo, said derivative having the formula:

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15 where X^1 and X^2 independently represent hydrogen, an acyl group or a hydrocarbyloxycarbonyl group, and where X^3 is an acyl group or a hydrocarbyloxycarbonyl group.

4. A compound according to claim 3 wherein the acyl group is a C_1 to C_{18} alkanoyl group.

5. A compound according to claim 3 wherein X^3 is an acyl group selected from the group consisting of formyl, acetyl, propanoyl and butanoyl.

6. A compound according to claim 3 wherein X^1 and X^2 both represent hydrogen.

7. A compound according to claim 6 wherein X^3 represents a C_1 to C_{18} alkanoyl group.

8. $1\alpha,25$ -dihydroxyvitamin D_3 -1,3,25-Triacetate.

9. $1\alpha,25$ -dihydroxyvitamin D_3 -1,3,25-Trihexanoate.

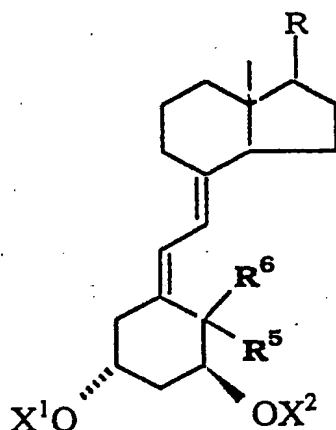
10. $1\alpha,25$ -dihydroxyvitamin D_3 -1,3,25-Trinonanoate.

11. $1\alpha,25$ -dihydroxyvitamin D_3 -25-Acetate.

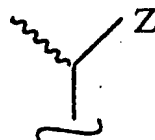
12. $1\alpha,25$ -dihydroxyvitamin D_3 -25-formate.

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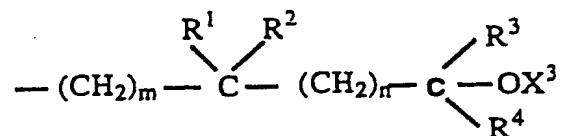
13. A method of providing for the regulated in vivo release of an active form of a vitamin D compound having a hydroxy group at carbon 25 which comprises modifying the vitamin D compound by derivatizing the hydroxy group at carbon 25 with an acyl group or a hydrocarbyloxycarbonyl group, to obtain a vitamin D derivative having the formula:



where R^5 and R^6 each represent hydrogen, or taken together R^5 and R^6 represent a methylene group, and where R is represented by the structure:



where the stereochemical center at carbon 20 in the side chain may have the R or S configuration, and where Z is selected from Y, -OY, -CH₂OY, -C \equiv CY and -CH = CHY, where the double bond may have the *cis* or *trans* stereochemical configuration, and where Y is selected from a radical of the structure:



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35 where m and n, independently, represent the integers from 0 to
5, where R^1 is selected from the group consisting of hydrogen,
OX⁴, fluoro, trifluoromethyl, and C₁₋₅- alkyl, which may be
straight chain or branched and, optionally, bear a hydroxy
substituent, and where R^2 is selected from the group consisting
40 of hydrogen, fluoro, trifluoromethyl and C₁₋₅ alkyl, which may be
straight-chain or branched, and optionally, bear a hydroxy
substituent, and where each of R^3 and R^4 , is selected from
trifluoromethyl and C₁₋₅ alkyl, which may be straight chain or
branched and optionally bear a hydroxy substituent, and where
45 R^1 and R^2 , taken together, represent an oxo group, or an
alkylidene group, $=CR^2R^2$, $=CR^2R^3$, or the group $-(CH_2)_p-$, where
p is an integer from 2 to 5, and where R_3 and R_4 , taken
together, represent the group $-(CH_2)_q-$, where q is an integer
from 2 to 5, and where X^1 , X^2 , and X^4 are each hydrogen, an acyl
50 group, or a hydrocarbyloxycarbonyl group, and where X^3
represents an acyl group or a hydrocarbyloxycarbonyl group.

14. The method of claim 13 wherein X^1 , X^2 , X^3 , and X^4
independently represent an acyl group or a
hydrocarbyloxycarbonyl group.

15. The method of claim 13 wherein X^3 is acyl or a
hydrocarbyloxycarbonyl group and X^1 , X^2 and X^4 each represent
hydrogen.

16. The method of claim 13 where X^1 , X^2 and X^4 are
each hydrogen or an acyl group, and X^3 is an acyl group.

17. The method of claim 16 where the acyl group is
acetyl.

18. The method of claim 16 where the acyl group is
propanoyl, butanoyl or hexanoyl.

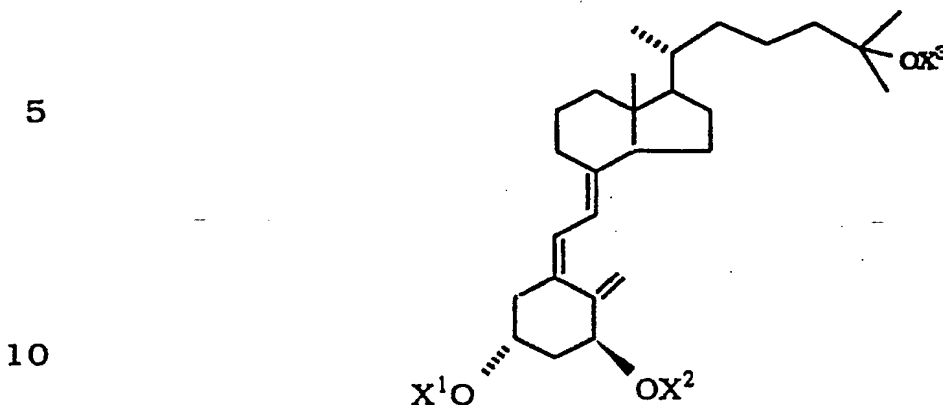
- 33 -

19. The method of claim 16 where each of X^1 , X^2 , X^3 and X^4 is acetyl.

20. The method of claim 16 where X^1 , X^2 and X^4 are each hydrogen and X^3 is acyl.

21. The method of claim 20 where X^3 is acetyl.

22. The method of claim 13 wherein said derivatized vitamin D compound has the formula:



where X^1 and X^2 are each hydrogen, an acyl group or a hydrocarbyloxycarbonyl group and where X^3 represents an acyl group or a hydrocarbyloxycarbonyl group.

23. The method of claim 22 where X^1 and X^2 are each hydrogen or an acyl group, and X^3 is an acyl group.

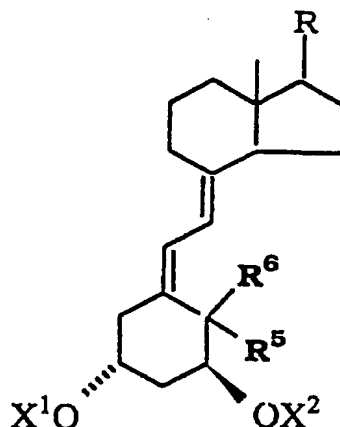
24. The method of claim 23 where the acyl group is acetyl.

25. In a method of treating disease states resulting from calcium metabolism disorders involving the administration of an active form of a vitamin D compound having a hydroxy group at carbon 25, the improvement comprising regulating the in vivo release of said active form of vitamin D compound by derivatizing the hydroxy group at carbon 25 with an acyl group or a hydrocarbyloxycarbonyl group to obtain a vitamin D derivative having the formula:

- 34 -

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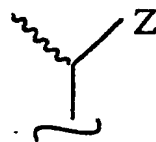
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where R^5 and R^6 each represent hydrogen, or taken together R^5 and R^6 represent a methylene group, and where R is represented by the structure:

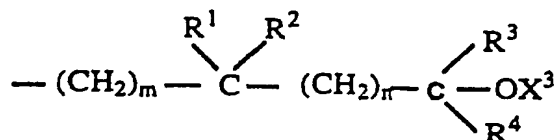
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where the stereochemical center at carbon 20 in the side chain may have the R or S configuration, and where Z is selected from Y , $-OY$, $-CH_2OY$, $-C \equiv CY$ and $-CH = CHY$, where the double bond may have the *cis* or *trans* stereochemical configuration, and where Y is selected from a radical of the structure:

35



40

where m and n , independently, represent the integers from 0 to 5, where R^1 is selected from the group consisting of hydrogen, OX^4 , fluoro, trifluoromethyl, and C_{1-5} -alkyl, which may be straight chain or branched and, optionally, bear a hydroxy substituent, and where R^2 is selected from the group consisting of hydrogen, fluoro, trifluoromethyl and C_{1-5} alkyl, which may be

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straight-chain or branched, and optionally, bear a hydroxy substituent, and where each of R^3 and R^4 , is selected from trifluoromethyl and C_{1-5} alkyl, which may be straight chain or
45 branched and optionally bear a hydroxy substituent, and where R^1 and R^2 , taken together, represent an oxo group, or an alkylidene group, $=CR^2R^2$, $=CR^2R^3$, or the group $-(CH_2)_p-$, where p is an integer from 2 to 5, and where R_3 and R_4 , taken together, represent the group $-(CH_2)_q-$, where q is an integer
50 from 2 to 5, and where X^1 , X^2 , and X^4 are each hydrogen, an acyl group, or a hydrocarbyloxycarbonyl group, and where X^3 represents an acyl group or a hydrocarbyloxycarbonyl group, and administering an effective dose of said vitamin D derivative to a subject.

26. The method of claim 25 wherein said derivatized compound is combined with a non-toxic pharmaceutically acceptable carrier and is administered in liquid form.

27. The method of claim 26 wherein said derivatized compound is administered by injection.

28. The method of claim 26 wherein said derivatized compound is administered intravenously.

29. The method of claim 26 wherein said derivatized compound is administered orally.

30. The method of claim 25 wherein said derivatized compound is combined with a non-toxic pharmaceutically acceptable carrier and is administered in solid form.

31. The method of claim 30 wherein said derivatized compound is administered by suppository.

32. The method of claim 30 wherein said derivatized compound is administered topically.

33. The method of claim 30 wherein said derivatized compound is administered orally.

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34. The method of claim 25 wherein said disease is selected from the group consisting of renal osteodystrophy, hypoparathyroidism, pseudohypoparathyroidism, hypocalcemia, osteomalacia, vitamin D-deficient rickets, postmenopausal osteoporosis, estrogen-lack osteoporosis, senile osteoporosis, steroid-induced osteoporosis, and psoriasis.

35. The method of claim 25 wherein said modified vitamin D compound is administered in an amount from about 0.5 µg/day to about 10 µg/day.

36. The method of claim 25 wherein at least two derivatized vitamin D compounds are administered to said subject to provide a spectrum of activity.

37. The method of claim 36 wherein one of said at least two derivatized vitamin D compounds is calcitriol in which the hydroxy group at carbon 25 is derivatized with an acyl group.

38. A method of modifying the in vivo activity profile of a biologically active vitamin D compound having hydroxy groups at carbons 1, 3 and 25, and, optionally, at other carbon positions, which comprises derivatizing the hydroxy group at carbon 25, and, optionally, one or more of the hydroxy groups at carbons 1, 3, or at said other positions in the molecule, with an acyl group or a hydrocarbyloxycarbonyl group.

39. The method of claim 38 wherein the active vitamin D compound is derivatized with an acyl group.

40. The method of claim 38 wherein one or more acyl group is CH₃CO.

41. The method of claim 38 wherein one or more acyl group is CH₃(CH₂)₄CO.

42. The method of claim 38 wherein one or more acyl group is formyl.

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43. The method of claim 38 wherein the in vivo activity profile is modified to illicit a more gradual onset or a more prolonged duration of biological response of said active vitamin D compound.

44. A method of regulating the in vivo availability of calcitriol comprising derivatizing the 25-hydroxyl group thereof with an in vivo hydrolyzable group.

45. The method of claim 44 wherein the hydrolyzable group is an acyl group.

46. The method of claim 44 wherein the hydrolyzable group is CH_3CO .

47. The method of claim 44 wherein the hydrolyzable group is $\text{CH}_3(\text{CH}_2)_4\text{CO}$.

48. The method of claim 44 wherein the hydrolyzable group is $\text{CH}_3(\text{CH}_2)_7\text{CO}$.

49. The method of claim 44 wherein the hydrolyzable group is a hydrocarbyloxycarbonyl group.

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FIG. 1

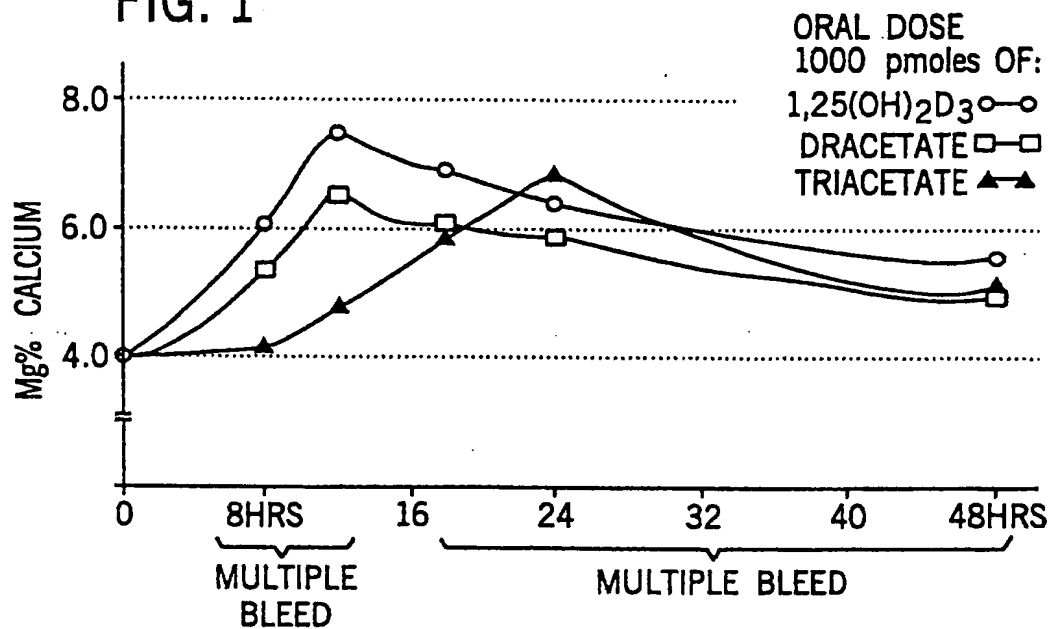
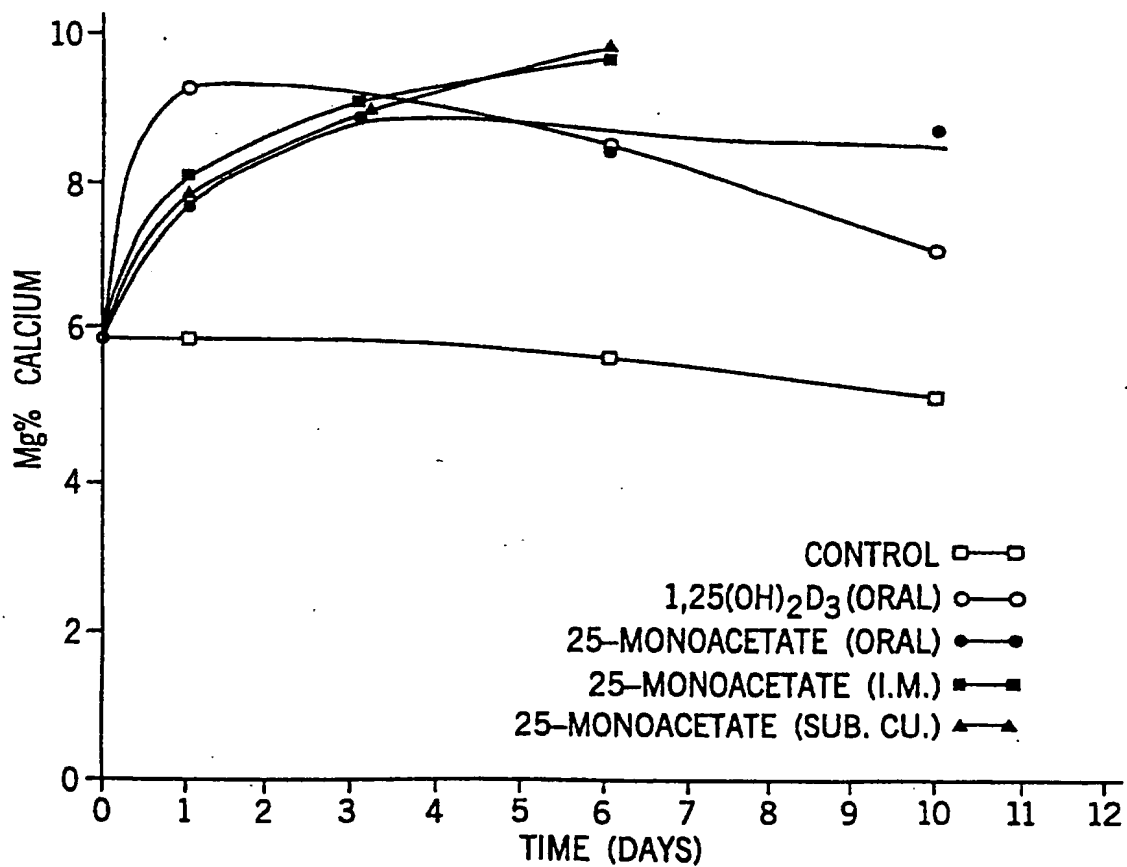


FIG. 2



SUBSTITUTE SHEET (RULE 26)

INTERNATIONAL SEARCH REPORT

International Application No

PL./US 96/15184

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07C401/00 A61K31/59

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C07C A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ENDOCRINOL. CALCIUM METAB. , 1982, pages 297-320, XP002019241 BORIS A ET AL: "Structure-activity relationships of compounds related to vitamin D" see the whole document ---	1-5,13, 16,17, 22-24, 38-40, 43-46
X	METAB. BONE DIS. RELAT. RES., vol. 1, no. 1, 1978, pages 23-28, XP002019242 BONJOUR J P ET AL: "Effect of synthetic vitamin D derivatives on calcium and phosphate metabolism in intact vitamin D-replete rats" see the whole document --- -/--	1-5,13, 16,17, 22-24, 38-40, 43-46

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

* Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
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T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

& document member of the same patent family

Date of the actual completion of the international search

22 November 1996

Date of mailing of the international search report

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Authorized officer

Watchorn, P

INTERNATIONAL SEARCH REPORT

International Application No

PL./US 96/15184

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JOURNAL OF STEROID BIOCHEMISTRY, vol. 13, no. 4, 1980, pages 409-413, XP002019243 MALLON J P ET AL: "Binding specificity of the rat serum vitamin D transport protein" see the whole document	1-5,13, 16,17, 22-24, 38-40, 43-46
X	--- CHEMICAL ABSTRACTS, vol. 121, no. 19, 7 November 1994 Columbus, Ohio, US; abstract no. 231159, TACHIBANA Y: "Preparation of 1-acyloxyvitamin D derivatives as pharmaceuticals" XP002019245 see abstract & JP 06 009 547 A (NISSHIN FLOUR MILLING CO;JAPAN) 18 January 1994 & "Formula Index, Volume 121, C25 H52 O-Z" 1994 , CHEMICAL ABSTRACTS , COLUMBUS OHIO, US see page 4084F, column 3, line 54 - line 56 see page 4135F, column 1, line 27 - line 30	1,2,13, 38-40, 43-46
X	--- CHEMICAL ABSTRACTS, vol. 121, no. 7, 15 August 1994 Columbus, Ohio, US; abstract no. 83752, Y. TACHIBANA ET AL: "Preparation of Vitamin D2 and Its Derivatives" page 1153; column 1; XP002019246 see abstract & JP 05 339 230 A (NISSHIN FLUOR MILLING CO.) 21 December 1993 & "Formula Index, Volume 121, C25 H52 O-Z" 1994 , CHEMICAL ABSTRACTS , COLUMBUS OHIO US see page 4084F, column 3, line 54-60 --- -/--	1,2,13, 38-40, 43-46

INTERNATIONAL SEARCH REPORT

International Application No

PC./US 96/15184

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CHEMICAL ABSTRACTS, vol. 117, no. 17, 26 October 1992 Columbus, Ohio, US; abstract no. 171825, Y. MORIMOTO ET AL: "Preparation of Vitamin D2 and D2 Derivatives and Intermediates" page 885; column 2; XP002019247 see abstract & JP 04 099 763 A (HOKUSAN K.K.) 31 March 1992	1-4,13, 22,23, 38,39, 43-45
X	CH 658 050 A (WISCONSIN ALUMNI RES FOUND) 15 October 1986 see examples 7,24	1-5,8, 13,14, 16,17, 22-24, 38-40, 42,46
X	FR 2 530 624 A (WISCONSIN ALUMNI RES FOUND) 27 January 1984 see page 6 - page 7	1,2,13, 38-40, 43-46
X	CHEMICAL ABSTRACTS, vol. 100, no. 19, 7 May 1984 Columbus, Ohio, US; abstract no. 156875, TEIJIN LTD: "1.alpha.,24(s),25-Trihydroxycholecalcifer ol" page 559; column 2; XP002019248 see abstract & JP 58 046 508 A (TEIJIN LTD) 17 October 1983	1,2,13, 14,16, 17,19, 38-40, 43-46
X	GB 2 026 494 A (WISCONSIN ALUMNI RES FOUND) 6 February 1980 see examples 15,16	1-7,11, 13,16, 17, 22-24, 38-40, 43-46
X	GB 2 023 146 A (UPJOHN CO) 28 December 1979 see claim 28	1-5,13, 38-40, 43-46

INTERNATIONAL SEARCH REPORT

International Application No

PL./US 96/15184

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>CHEMICAL AND PHARMACEUTICAL BULLETIN, vol. 30, no. 12, 1982, TOKYO JP, pages 4297-4303, XP002019244 Y. KOBAYASHI ET AL: "Studies on Organic Fluorine Compounds. XXXIX. Studies on Steroids. LXXIX. Synthesis of 1.alpha.,25-Dihydroxy-26,26,26,27,27,27-he xafluorovitamin D3" see page 4302, paragraph 3 -----</p>	<p>1,2, 38-40, 43-46</p>

INTERNATIONAL SEARCH REPORT

national application No.

PCT/US 96/15184

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 25-37
because they relate to subject matter not required to be searched by this Authority, namely:
Remark : Although claims 25-37 are directed to a method of treatment of the human/animal body the search has been carried out and based on the alleged effects of the compounds.
2. ☒ Claims Nos.: 38-49
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see annex
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US 96/ 15184

FURTHER INFORMATION CONTINUED FROM PCT/ISA/210

Claims 38-49 refer to "derivatisation at C-25" of "a biologically active Vitamin D compound" neither of these unclear terms is defined in the claims and no characterising structured feature can be recognised in these claims which are rendered so unclear that a full search is not possible - Art.6 PCT/ Art.17 (2)(a)(ii) PCT and Art.17 (2)(b) PCT. Claims 38-49 were searched in as far as they are commensurate in scope with claim 1.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PC., US 96/15184

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
CH-A-658050	15-10-86	US-A- 4195027	25-03-80
		AU-B- 525781	02-12-82
		AU-A- 4339479	26-07-79
		BE-A- 873512	16-05-79
		BE-A- 877356	15-10-79
		CA-A- 1156251	01-11-83
		CH-A- 653321	31-12-85
		DE-C- 2933189	29-05-91
		DE-C- 2954557	11-07-91
		DK-B- 169871	20-03-95
		DK-B- 170437	04-09-95
		FR-A- 2438035	30-04-80
		FR-A- 2446278	08-08-80
		FR-A- 2436140	11-04-80
		FR-A- 2478631	25-09-81
		FR-A- 2529888	13-01-84
		GB-A,B 2013686	15-08-79
		JP-C- 1268975	10-06-85
		JP-A- 58135855	12-08-83
		JP-B- 59045673	07-11-84
		JP-C- 1268976	10-06-85
		JP-A- 58135856	12-08-83
		JP-B- 59045674	07-11-84
		JP-C- 1268977	10-06-85
		JP-A- 58135857	12-08-83
		JP-B- 59045675	07-11-84
		NL-A- 7900331	18-07-79
		NL-A- 7905040	17-07-80
		SE-B- 429971	10-10-83
		SE-A- 7907631	13-09-79
		WO-A- 7900513	09-08-79
		CA-A- 1254225	16-05-89
FR-A-2530624	27-01-84	US-A- 4428946	31-01-84
		AU-B- 571846	28-04-88
		AU-A- 1826383	23-02-84
		BE-A- 897377	14-11-83
		BE-A- 897378	14-11-83
		CA-A- 1225593	18-08-87
		DE-T- 3390126	10-01-85

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 96/15184

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
FR-A-2530624		FR-A- 2530470	27-01-84
		GB-A, B 2124080	15-02-84
		GB-A, B 2126234	21-03-84
		NL-T- 8320229	01-06-84
		WO-A- 8400549	16-02-84
		AU-A- 1821383	23-02-84
		CH-A- 661047	30-06-87
		DE-T- 3390125	20-09-84
		NL-T- 8320218	01-06-84
WO-A- 8400550	16-02-84		

GB-A-2026494	06-02-80	US-A- 4188345	12-02-80
		AU-B- 521131	18-03-82
		AU-A- 4920679	31-01-80
		BE-A- 877918	16-11-79
		CH-A- 641445	29-02-84
		CH-A- 650237	15-07-85
		FR-A- 2433514	14-03-80
		NL-T- 7920012	30-05-80
		NL-T- 7920016	30-05-80
		SE-A- 8001746	05-03-80
		SE-A- 8001747	05-03-80
		US-A- 4224230	23-09-80
		US-A- 4263214	21-04-81
		US-A- 4229357	21-10-80
		US-A- 4229358	21-10-80
		US-A- 4226787	07-10-80
		WO-A- 8000338	06-03-80
		WO-A- 8000339	06-03-80

GB-A-2023146	28-12-79	US-A- 4206131	03-06-80
		CH-A- 647760	15-02-85
		DE-A- 2923953	03-01-80
		FR-A- 2443459	04-07-80
		JP-A- 55002686	10-01-80
		NL-A- 7904585	21-12-79
		US-A- 4267117	12-05-81
